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Commentary

Nongenotoxic Carcinogens: An Extension of the Perspective Provided by Perera

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Perera recently discussed the very real problems that accompany any attempt to classify rodent carcinogens into two groups—genotoxic or nongenotoxic. Not the least of these problems is that no agreed definition of these two terms exist. Nonetheless, the current carcinogen databases, for example, that of the U.S. National Toxicology Program (NTP), clearly comprise two broad groups of carcinogens—DNA reactive, mutagenic and multiply carcinogenic chemicals, and others. The others appear to be nonreactive to DNA, are inactive in the primary mutagenicity assays, and usually elicit highly selective carcinogenic responses in animals. These two classes of carcinogen are illustrated by examples taken from the NTP database and are discussed within the possible context of the latter group not being active in humans or, if they are, only when a threshold dose has been exceeded, chronically.

Perera (*1*) recently discussed uncertainties associated with the categorization of rodent carcinogens for purposes of risk assessment, according to their presumed mechanism of action—genotoxic or nongenotoxic. These uncertainties call into question automatic secondary assumptions such as that nongenotoxic carcinogens operate only above a certain threshold dose and are of reduced hazard to humans. Perera concluded her analysis by stating that, in light of these uncertainties and in the absence of evidence to the contrary, there is currently no convincing scientific rationale for assigning a greater or lesser degree of risk to carcinogens based on their presumed mechanism or stage of action (*1*). The direct consequence of this conclusion is that all rodent carcinogens must be regarded as posing an equal hazard to humans once adjustment has been made for the differing dose levels used in the defining rodent bioassays.

Much of what Perera wrote could be justified by the fact that to date no single definitions of the terms "nongenotoxic" or "tumor promoter" have emerged. In the absence of such agreed definitions it could be argued that it is pointless to proceed further. Set against that viewpoint is the one held by many investigators that nongenotoxic mechanisms of carcinogenicity are strongly indicated but imperfectly established at present (*2–4*). Further, it would be expected that some nongenotoxic mechanisms of carcinogenicity in rodents may not apply to humans and that some may be threshold related. The latter viewpoint currently acts as the stimulus for much basic research and is therefore worthy of equal consideration.

Probably the least useful exercise to attempt at present is a definition of the term "nongenotoxic." Rather, it is worth defining areas of common assent and identifying areas of apparent disagreement, the latter of which usually turn out to be areas where data are missing, which in turn allows opinions to dominate.

If there is any common ground, it must be that the first five rodent carcinogens shown in Table 1 are not only genotoxic but that their genotoxicity is mechanistically related to their rodent carcinogenicity. The first two of these carcinogens are active at the site of initial contact in the rodents, and they are each active in both sexes of both species. It therefore seems probable that similar effects would be observed in humans exposed to these chemicals, and further, that compelling data would be required to counter the assumption of the absence of a threshold dose level. These two examples therefore provide a perfect case for risk estimation, i.e., one could calculate a cancer incidence of, say, 1 in 10^6 in man and be as confident as ever one

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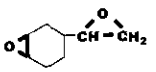
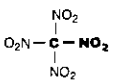
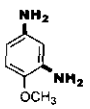
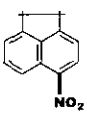
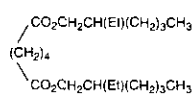
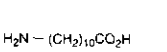
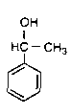
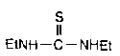
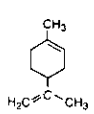
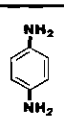
Table 1. Carcinogenicity data for B6C3F$_1$ mice and F344 rats, as reported by NTP and extracted by Ashby and Tennant (5).[a]

CAS No NTP Tech Report No (Year) Chemical Structure (alerting substructure in bold) Chemical Name	Structural alert	Salmonella assay response (Zeiger)	Route	Maximum dose (ppm) Rats	Maximum dose (ppm) Mice	Tumor site	Tumor data identified in Summary of NTP Technical Report % Tumor-bearing animals Rats Male C	Rats Male L	Rats Male H	Rats Female C	Rats Female L	Rats Female H	Mice Male C	Mice Male L	Mice Male M	Mice Male H	Mice Female C	Mice Female L	Mice Female M	Mice Female H
106-87-6 362(1989)  4-Vinyl-1-cyclohexene Diepoxide	+	+	*Skin*	*30 mg/ animal*	*10 mg/ animal*	Skin Ovary Lung	0	66	72	0	32	68	0 0	28 0	78 0	84 0	0 2 8	12 0 18	74 35 22	82 36 14
509-14-8 386 (1990)  Tetranitromethane	+	+	Inhalation	0.0005	0.0002	Lung	2	66	92	0	44	100	24	52		92	8	48		98
39156-41-7 84(1978)  2,4-Diaminoanisole Sulfate	+	+	Food	0.5	0.24	Clitoral gland Preputial gland Skin Thyroid gland Zymbal's gland	C_L C_H L H 0 0 4 16 0 0 4 14 5 0 4 35 0 0 2 16			C_L C_H L H 0 6 10 16 5 2 2 20 0 0 0 14			C_L C_H L H 2 0 0 24				C_L C_H L H 0 0 0 18			
602-87-9 118(1978)  5-Nitroacenaphthene	+	+	Food	0.24	0.12	Clitoral gland Liver carcin. Lung *Mammary gland* Ovary Skin	C_L C_H L H 0 2 14 0 2 0 50 43			C_L C_H L H 0 0 13 13 0 2 17 10 0 0 11 15 0 0 55 74							C L H 4 49 95 0 10 19			
107-06-2 55(1978)  1,2-Dichloroethane	+	+	Gavage	95	299	Circulatory Lung Mammary gland Stomach carcin. Subcutaneous Uterus	C_P C L H 2 0 18 14 0 0 6 18 0 0 10 12			C_P C I H 2 0 2 36			C_P C L H 0 0 2 31				C_P C I H 3 5 14 31 0 0 18 15 0 0 10 11			
103-23-1 212(1982)  Di(2-ethylhexyl)adipate	—	—	Food	2.5	2.5	Liver adenomas Liver carcin.							26	41		55	6 2	38 28		37 24
2432-99-7 216(1982)  11-Aminoundecanoic Acid	—	—	Food	1.5	1.5	Liver adenomas Urinary bladder	2 0	18 0	16 14											
98-85-1 369(1989)  α-Methylbenzyl Alcohol	—	—	Gavage	750	750	Kidney	0	4	10											
105-55-5 149(1979)  N,N'-Diethylthiourea	—	—	Food	0.025	0.05	Thyroid gland	0	2	23	0	9	37								
5989-27-5 *347(1990)*  *d*- Limonene	—	—	Gavage	600	1000	Kidney	0	16	22											
624-18-0 174(1979)  *p*-Phenylenediamine.2HCl	+	+	Food	Non-carcinogenic																

Abbreviations: C, control; L, low-dose groups; M, mid-dose groups; H, high-dose groups; Cp, pooled controls.

[a]The duration of the negative study was 103 weeks.

can be that such an incidence of induced cancer could be detected by an appropriately sensitive epidemiological study.

Similar arguments would apply to the next three rodent carcinogens in Table 1, albeit an element of uncertainty is caused in these cases by the seemingly random spread of affected tissues. However, genotoxic carcinogenesis is far from understood at the level of organotropic responses, so such concerns remain minor. In summary, the first five carcinogens shown in Table 1, which are representative of the majority of the National Toxicology Program (NTP) carcinogen database, can be safely assumed to present a commensurate carcinogenic hazard to man. These five carcinogens were selected because they are active in all four test groups and at both the low and the high bioassay dose levels [this was done to eliminate from this discussion the important but secondary issues of species-specific metabolism or high-dose toxicity influencing carcinogenicity (*5*)]. The genotoxic noncarcinogen shown at the bottom of Table 1 was also included to introduce and then to dismiss from this discussion the concept of genotoxic noncarcinogens (*4*).

The second set of five rodent carcinogens shown in Table 1 is also from the NTP carcinogen database and was selected to illustrate the type of agent currently (albeit sometimes loosely) referred to as nongenotoxic carcinogens. The impression given by these five carcinogens is that they are grossly different from the first five. In particular, the prospect is raised that the biological activities of these agents in the tissues subject to their selective carcinogenicity are probably a stronger lead to their carcinogenicity than is any selective DNA damaging (genotoxic) activities they may unexpectedly show in those tissues. In particular, even in the absence of any mechanistic data, one would be less confident than with the first five carcinogens in predicting a human cancer incidence of 1 in 10^6 for people exposed to low dose levels of limonene, for example. It is suggested that this gross feeling is the primary stimulus for studies into the mechanism of nongenotoxic carcinogens—it cannot be precisely defined, but it would probably be negligent to ignore such a strong indication. In fact, mechanistic data exist, in various stages of refinement, to support possible alternative (nongenotoxic) mechanisms of action for these agents (*2*).

Having failed to define the term nongenotoxic herein, it is necessary to address the obvious counter arguments at this point. Although classed as structurally nonalerting, the second five carcinogens shown in Table 1 may contain hitherto unrecognized electrophilic centers. Further, although nonmutagenic to Salmonella, they will probably be active in one or other additional *in vitro* mammalian cell genotoxicity assays (all chemicals are active, without exception, when a sufficient number of tests have been conducted). Finally, each chemical may directly modify the DNA of the affected tissues. These counter arguments are accepted as possible, but they do not appear to be strong enough to suggest that the 10 carcinogens in Table 1 represent a mechanistic continuum. The only point made is that it seems curious that those carcinogens that are most selective in their carcinogenic activity should also be the ones that have novel electrophilic sites and subtle genotoxic activities.

If it is accepted as possible that a mechanistic gulf separates the first five carcinogens in Table 1 from the second five, then there is an urgent need to focus research to answer the questions posed or implied by Perera (*1*), as follows.

First is the need for at least one agreed precedent for each of the several classes of nongenotoxic carcinogens. For example, progress in the study of peroxisome proliferators (e.g., the hepatic carcinogen shown sixth in Table 1) is being slowed by each of the major research groups studying a different member of the class. This delays overall progress and complicates comparison of data. A similar situation exists in the study of male rat renal carcinogens operating via the α-2-microglobulin mechanism. Selection of a single agent for joint study in each class of nongenotoxic carcinogen is therefore suggested.

Second, if an agent is to be considered as a possible nongenotoxic carcinogen, it should first be evaluated for genotoxicity in the standard genetic toxicity assays. At present this need is usually neglected. Thus, dimethylpentane, a renal carcinogen associated with α-2-microglobulin, is devoid of any published genotoxicity data despite the advanced stage of mechanistic studies on it. Likewise, few bone marrow cytogenetic assay data have been reported for the peroxisome proliferators and none for the male rat renal carcinogen limonene. The activity of a presumed nongenotoxic carcinogen in a genotoxicity assay does not automatically exclude a nongenotoxic mechanism of carcinogenic action, but such data should be available for consideration.

Third is the urgent need to demonstrate unequivocally a threshold effect for at least one presumed nongenotoxic carcinogen. Such a carcinogenicity bioassay could be conducted in a single sex of a single species, but it would have to be designed such that agreement was obtained in advance regarding its statistical resolving power. If an acute precursor event is known to be directly involved in the carcinogenicity of the agent selected for study, threshold studies could be related initially to that event, much as Swenberg and his colleagues (*6*) have done when studying the mechanism of action of limonene as a renal carcinogen using renal foci promotion studies. However, some knowledge of the relevance of the precursor event to carcinogenicity would be necessary. The threshold studies by Lucier and Portier (*7*) on TCDD illustrate both the promise and the potential complexity of this approach (the extreme and unrepresentative metabolic stability of TCDD confuses further the threshold issue in this case). At present, however, there are no statistically sound data on thresholds in nongenotoxic carcinogenesis.

A particularly illuminating example of how debates on nongenotoxic rodent carcinogenesis can become confused by secondary issues is provided by the U.S. Food and Drug Administration (FDA) consideration of the case of the color FD&C No. 3 (*8*). This chemical induces follicular cell tumors in male Charles River CD-1 rats when dosed at a concentration of 4% in the diet. From the detailed discussion provided in the U.S. Federal Register (*8*), it becomes

clear that the extensive evidence available is in favor of this chemical being carcinogenic by virtue of hyperstimulation of the thyroid, rather than by it directly damaging thyroid cell DNA. However, this well-developed argument in favor of a nongenotoxic mechanism of action was confused, and finally rejected, based on two secondary issues: *a)* discussion of whether the agent is truly inactive in the available genetic toxicity assays and *b)* whether a threshold dose had been established for the thyroid effects. The concerns expressed by the FDA on these two secondary issues were justified. What seems to be unjustifiable was that the short-term need to be able to register this agent as being absolutely without a human carcinogenic hazard obscured the wealth of data supporting a nongenotoxic mechanism of rodent carcinogenicity. Given the strength of this mechanistic data, the question of whether FD&C No. 3 is inactive in the genotoxicity assays conducted becomes almost irrelevant. For example, even if more extensive repeat tests conducted in the mouse lymphoma L5178Y assay were to uncover a positive test response, it seems unlikely that this would affect the conclusion of a nongenotoxic mechanism of thyroid carcinogenicity. Likewise, further data may unequivocally establish a threshold dose for the hormonal and carcinogenic thyroid effects reported, but the absence of such data does not weaken the central strong implication of a nongenotoxic mechanism of carcinogenic action.

There are therefore two alternative positions to adopt in response to the 10 carcinogens shown in Table 1. The first is to assume that each is of similar hazard to man. The second is to consider each carcinogen within the context of its total biology and to assess the extent to which its carcinogenicity may apply to humans. Consideration of all of the available data will usually enable the agent to be classified tentatively as operating by a genotoxic or a nongenotoxic mechanism. Once a possible nongenotoxic mechanism is indicated, further studies become justified to study the relevance of its carcinogenicity to man and/or the possible existence of a threshold dose.

The term "nongenotoxic carcinogen" therefore holds a similar position to that held by the word "evolution" in the 1890s when the Marquis of Salisbury defined it as "an indefinite word which has the gift of alleviating so many perplexities and masking so many gaps in our knowledge" (*9*). At present its main use, while remaining tentative, is to aid priority setting in carcinogen detection and regulation (*10,11*), as recently discussed by Goodman and Wilson (*12*). Goodman and Wilson (*12*) actually extended this present discussion by suggesting that chemicals should not be classified as carcinogens and noncarcinogens, but rather that we should assume all chemicals are carcinogenic and that some have too low a potency to produce a statistically significant increase in tumors with a given experimental protocol. That proposal was discussed by Goodman and Wilson (*12*) within the single context of potency, but a more general validity may be established by evaluating the present NTP noncarcinogens in a wider variety of rodent species and strains. Thus, a characteristic of nongenotoxic carcinogenesis tissue bioassays is that they tend toward species/strain/sex/tissue specificity (*4*), so the greater the amount of different bioassays conducted on a noncarcinogen, presumably the greater will be the chance of an isolated carcinogenic response being observed. However, if one first dissolves the boundary between genotoxic and nongenotoxic carcinogens (*1*) and then the one between carcinogens and noncarcinogens (*12*), Table 1 becomes a continuum of hazardous chemicals. If to this is added the counsel of perfection that we should "consider the multiplicity of action of a single agent and the influence of all agents to which humans are exposed simultaneously" (*1*), then any practical steps toward carcinogenic regulation are proscribed.

It is therefore proposed that the terms "genotoxic"* and "nongenotoxic" carcinogen should be maintained and refined in the causes of research into mechanisms of carcinogenicity (*13,14*) and of efficient human carcinogenic hazard assessment. For the present this is subjectively supported by the suggestion that any system that fails, for example, to accord tetranitromethane (Table 1) an intrinsically higher (i.e., dose independent) potential human hazard rating that limonene (Table 1) must be regarded as deficient. Nonetheless, the reservations expressed by Perera (*1*) are valid and therefore use of these terms must, for the present, remain tentative.

*Practical methods to screen for genotoxins are discussed by Ashby and Morrod (*3*).

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